

WHAT IS CLAIMED IS:

1. An adenoviral vector gene delivery system comprising:
 - (a) a helper dependent adenovirus vector, hdAd, comprising a genome substantially devoid of adenoviral protein coding sequences, but encoding a gene and expression control sequences, the expression of which in a recipient cell is desired;
 - (b) helper adenoviruses of different serotypes encoding all functions required to facilitate hdAd genome packaging and replication, but which helper adenoviruses themselves do not package into infectious viral particles; and
 - (c) a cell into which may be introduced, in separate introduction steps, a helper adenovirus of a first serotype and said hdAd, such that each said separate introduction step results in the production of a packaged hdAd having the serotype of the helper adenovirus co-introduced into said cell in said step.
2. The adenoviral vector gene delivery system of claim 1 wherein said helper adenoviruses of different serotypes are serotype 2 and serotype 5.
3. The adenoviral vector gene delivery system of claim 1 wherein each said helper adenovirus comprises a packaging signal flanked on either side by at least one lox site.
4. The adenoviral vector gene delivery system of claim 3 wherein Cre recombinase is expressed in said cell.
5. A method for repeatedly introducing into a living organism a gene in a viral vector, wherein expression of said gene is desired in said living organism under conditions whereby immune responses induced against said viral vector by a first administration of said viral vector have little or no inhibitory effect upon each repeat introduction of said gene, comprising the steps of:
 - (a) making a series of helper adenoviruses of differing serotypes;
 - (b) making a helper dependent adenovirus vector, hdAd, having a genome encoding said gene, an

7 adenoviral packaging signal, the adenoviral left ITR and the adenoviral right ITR and as much
8 additional nucleic acid sequences as are necessary to ensure efficient expression of said gene and
9 efficient packaging of said hdAd genome, but encoding little or no adenoviral gene products;
10 (c) generating a first stock of said hdAd *in vitro* by co-introducing into a cell said hdAd genome
11 and a helper adenovirus of a first serotype under conditions whereby essentially no infectious
12 particles of helper virus are present in the final hdAd stock, but wherein said stock is highly
13 enriched in infectious particles comprising said hdAd genome and capsid proteins encoded by
14 said helper adenovirus of said first serotype;
15 (d) repeating step (c) as many times as desired using a helper adenovirus of a different serotype
16 each time said step (c) is repeated, such that a series of infectious hdAd stocks are generated,
17 with each said stock having a capsid of different serotype; and
18 (e) sequentially introducing into said living organism portions of said infectious hdAd stocks
19 having a capsid of different serotype.

6. The method according to claim ~~5~~ wherein each said helper adenovirus comprises a packaging
signal flanked on either side by at least one lox site.

7. The method according to claim 6 wherein Cre recombinase is expressed in said cell.

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8. A kit comprising:

- 2 (a) an hdAd vector encoding a gene under control of a transcriptional promoter within a genome
3 comprising an adenoviral right ITR, an adenoviral left ITR and an adenoviral packaging
4 sequence;
5 (b) a series of helper adenoviruses of different serotype.

9. The kit according to claim 8 wherein each said helper adenovirus has a genome comprising an
adenovirus packaging signal flanked on either side by a lox site.

10. A method for repeat administration of a gene to a living organism, comprising making adenoviral vectors wherein the capsid protein serotype of said vector is altered prior to each said repeat administration, such that immune responses induced in said living organism against said adenoviral vector do not limit expression of said gene due to said adenoviral vector having a capsid serotype that is different upon each repeat administration.

11. The method according to claim 10 wherein said gene encodes an immunogenic gene product against which immune responses are induced in said living organism.

12. The method according to claim 10 wherein said gene encodes a function which corrects a genetic defect present in said living organism or which encodes a therapeutic product for treatment of a disease condition in said living organism.

Sub C² 13. A method of making a series of genetically identical adenoviral vectors wherein each member of said series has a different serotype, for delivering and expressing a desirable gene in a recipient of said series of genetically identical adenoviral vectors which comprises:

- (a) making a series of helper adenoviruses of differing serotypes;
- (b) making a helper dependent adenovirus vector, hdAd, having a genome encoding said gene, an adenoviral packaging signal, the adenoviral left ITR and the adenoviral right ITR and as much additional nucleic acid sequences as are necessary to ensure efficient expression of said gene and efficient packaging of said hdAd genome, but encoding little or no adenoviral gene products;
- (c) generating a first stock of said hdAd *in vitro* by co-introducing into a cell said hdAd genome and a helper adenovirus of a first serotype under conditions whereby essentially no infectious particles of helper virus are present in the final hdAd stock, but wherein said stock is highly enriched in infectious particles comprising said hdAd genome and capsid proteins encoded by said helper adenovirus of said first serotype;
- (d) repeating step (c) as many times as desired using a helper adenovirus of a different serotype each time said step (c) is repeated, such that a series of infectious hdAd stocks are generated, with each said stock having a capsid of different serotype; and

17 (e) recovering said infectious hdAd stocks ~~having a capsid of different serotype to obtain said~~
18 ~~series of~~ genetically identical adenoviral vectors.

1 14. A series of genetically identical adenoviral vectors wherein each member of said series has a
2 different serotype produced according to the method of claim 13.

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